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54 Title of Invention: Method of Manufacturing Isoflavone Compounds

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SPECIFICATIONS

**1. Title of the Invention**

Method of Manufacturing Isoflavone Compounds

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**2. Claims**

A method of manufacturing isoflavones, such method characterised in that, when isoflavone compounds are manufactured from soybean extract solution or soybean meal, the soybeans or soybean meal is heated to between 45°C and 55°C during any one, or a plurality of, the 10 soaking process, the grinding process, or the enzyme reaction process after the grinding process, in such a manner as to maximise the  $\beta$ -glucosidase activity in the soybeans.

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**3. Detailed Description of the Invention**

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**Applicable field of industry**

The present invention relates to a method of manufacturing isoflavone compounds and more particularly, isoflavone compounds that are rich in aglycones, from soybeans.

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**Prior art, and deficiencies thereof**

Soybeans contain isoflavone compounds such as daidzin, clycitin, genistin, daidzein and genistein and so forth, and these are known to have such physiologically active effects as oestrogen effects, antioxidant action, antihaemolytic action, antibacterial action, anti-lipaemic action, and anti-cholesterol effects and so forth, and moreover, such isoflavone compounds 25 have been recognised recently as possessing such cancer-controlling effects as inducing the differentiation of cancer cells and preventing the cancer genes and so forth, such that the value of such isoflavone compounds has received great attention.

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Such cancer-controlling and other pharmaceutical effects of the isoflavone compounds are not 30 due to glycosides, but principally to such aglycones as daidzein and genistein and the like.

JP 62-126186 for example reveals a method of deriving isoflavone compounds from soybean extract solution, but because at least 95% of the isoflavones present in soybeans are present as glycosides, the isoflavone compounds derived by this method are principally glycosides, and only very small amounts of the aglycones are produced.

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**Means employed in order to overcome such deficiencies**

The inventors of the present invention investigated methods of deriving at low cost and in large volumes the aglycones that are the extremely valuable portion of the isoflavone compounds, and discovered that the action of  $\beta$ -glucosidase in soybeans readily breaks down the sugar bonds in the isoflavones in soybeans and converts such isoflavones to aglycones, and that such conversion reaches a maximum at a temperature of 50° C and a pH of 6.3.

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The present invention was developed from this discovery, and is a method of manufacturing isoflavones, such method characterised in that, when isoflavone compounds are manufactured from soybean extract solution or soybean meal, the soybeans or soybean meal is heated to between 45° C and 55° C during any one, or a plurality of, the soaking process, the grinding process, or the enzyme reaction process after the grinding process, in such a manner as to maximise the  $\beta$ -glucosidase activity in the soybeans.

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The following is a more detailed description of the present invention.

The soybeans that form the raw material may be in any form, provided only that the enzymes therein have not been deactivated, and such forms as minimally denatured de-fatted soybeans, soybean meal, shelled soybeans and whole soybeans and so forth may be employed.

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In order to manufacture the isoflavone compounds from the extract solution from such soybeans, the soybeans are soaked in from five to ten times as much warm water at a temperature of from 45° C to 55° C, and the soaking water forms the extract solution which is the raw material for refining, while because the proportion of daidzein in such soaking water

is approximately 10% higher than that in soybeans, such soaking water forms an excellent raw material for refining.

Moreover, when soybean meal is employed for the manufacture of the isoflavones, soybeans that have been soaked in the manner described above may be ground together with the soaking water, or the soybeans may be soaked in water at room temperature and the soaked soybeans may then be ground at between 45° C and 55° C, or the soybeans may be heated to between 45° C and 55° C after grinding, in order to bring on the enzyme reaction, but preferably the soaking is conducted at from 45° C to 55° C and the beans are ground together with the soaking water at from 45° C to 55° C, with the meal so obtained being held at a temperature of from 45° C to 55° C for several hours. In this manner, the proportion of the isoflavones in the soybeans is greatly increased.

The soybean extract solution or soybean meal obtained in this manner is employed as the raw material for refining, but there are two methods of refining the isoflavone compounds.

One method involves refining by solvents. In this method, the raw materials for refining, namely, the soybean soaking water or the soybean meal, is powdered by drying in hot air or by freeze drying and the like, the powder so obtained is de-fatted by means of n-hexane or petroleum ether and the residue is dried and then extracted, whereby isoflavone aglycones only are obtained.

In the second method, the isoflavone compounds that are contained in the powdered raw materials for refining are reflux extracted by means of hydrated alcohol, the extract solution so obtained is concentrated and dried by known art, the residue is dissolved in a small amount of hydrated alcohol and adsorbed to a reverse phase type resin such as for example YMC-GEL ODS-A Type 60-01 (manufactured by Yamamura Kagaku Kenkyujo KK) or *Diaion HP-20* (manufactured by Mitsubishi Chemical Ltd) and the like, whereupon the resin is thoroughly rinsed with water, the phenolic acid is eluted with 20% hydrated alcohol, and isoflavone compounds rich in aglycones are separated and produced by means of 80% hydrated alcohol.

Moreover, the aglycone fraction only may be obtained by flushing the glycoside fraction with approximately 40% hydrated alcohol and elution with 80% hydrated alcohol.

5 The reverse phase resins employed in this case may be readily rinsed and regenerated with organic solvents such as for example alcohols or acetones and the like, and may then be reused.

10 Moreover, the raw materials for refining may be refined by direct adsorption to the resin, without the raw materials being powdered. Soaking water may be brought into contact with the reverse phase resins without further processing, or meal may be filtered by known art and the filtrate, or supernatant produced by centrifugal separation, may be brought into contact with the reverse phase resins.

15 The following describes examples of experiments relating to the present invention, and describes the effects thereof.

#### Experiment 1

Shelled soybeans were soaked for 6 hours in five times their own volume of water at between 20° C and 80° C, the soaked soybeans and the soaking water were cooled directly and frozen. 20 The soaked soybeans and soaking water were dried in a freeze dryer and then powdered, a fixed amount was reflux extracted with 80% methanol, and a fixed amount of the measured material was analysed by high-speed liquid chromatography (Waters Model 209 D).

The results are set out in Table 1.

Table 1

Soaking temperature	Proportion of aglycones (%)			
	Soaked soybeans + soaking water		Soaking water	
	Genistein*	Daidzein**	Genistein*	Daidzein**
20 (° C)	3	5	-	-
30	8	8	-	-
40	14	16	20	36
45	18	24	23	39
50	23	29	24	40
55	21	26	33	45
60	12	15	18	36
70	6	7	-	-
80	4	4	-	-

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$$\frac{\text{Genistein (mg)}}{\text{Genistein (mg)} + \text{Genistin (mg)}} \times 100$$

\*\* 
$$\frac{\text{Daidzein (mg)}}{\text{Daidzein (mg)} + \text{Daidzin (mg)}} \times 100$$

### Experiment 2

Shelled soybeans were soaked for 2 hours in five times their own volume of warm water at 10 50° C that had been adjusted to a pH of between 5.5 and 11; the soaking water was acidified, and immediately filtered with a 0.45 µm filter, and analysed by high-speed liquid chromatography, in the same manner as for Experiment 1.

The results are set out in Table 2.

Table 2

Soaking water pH	Proportion of aglycones (%)	
	Genistein	Daidzein
5.5	33	42
6.3	39	43
6.9	35	39
8.0	33	35
9.0	19	23
10.0	17	20
11.0	15	16

As is evident from the results of Experiments 1 and 2, the maximum conversion to isoflavone aglycones is achieved at a soaking temperature of between 45° C and 55° C and a soaking water pH of 6.3.

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### **Practical Embodiments**

The following describes practical embodiments of the present invention.

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#### **Practical Embodiment 1**

5 kg of shelled soybeans are soaked for 2 hours in 25 l of warm water that is maintained at 50° C.

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Next, the soaking water is concentrated in an evaporator and dried, to yield 450 g of refining raw material. This is de-fatted by means of n-hexane, using a Soxhlet's extractor. The residue is then dried thoroughly and extracted by means of ethyl ether to yield 0.5 g of isoflavone aglycones.

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#### **Practical Embodiment 2**

Shelled soybeans that have been soaked as in Practical Embodiment 1 are ground together with their soaking water at 50° C, allowed to stand for 1 hour, and are then dried in a freeze dryer and powdered, to yield 4.1 kg of refining raw material. This is de-fatted by means of n-hexane, and the aglycones are extracted by means of ethyl ether, in the same manner as for Practical Embodiment 1, to yield 7.2 g of isoflavone aglycones. The yield of aglycones is improved by 60% by grinding at 50° C and by standing at that temperature for 1 hour.

#### **Practical Embodiment 3**

50 l of water at a temperature of 50° C is added to 10 kg of minimally denatured de-fatted soybeans (Nissei Soya Flour) and stirred for 1 hour. This is hot air dried by spray drying to

yield the refining raw material. The isoflavones are extracted by means of five times the volume of the refining raw material of hot 80% methanol and dried under reduced pressure to yield 103 g of a raw isoflavone fraction. This is redissolved in a small amount of methanol and passed through a  $\phi$  70 mm x 100 cm column packed with ODS-A Type 60-01 (manufactured by Yamamura Kagaku Kenkyujo KK) as the packing material and adsorbed.

Next, the phenolic acid and isoflavone glycosides are extracted by means of 40% methanol and discarded, and the residue is eluted by means of 80% methanol and dried under reduced pressure, to yield 9.5 g of aglycones.

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#### Practical Embodiment 4

1 kg of *Diaion* HP-20 synthetic adsorbent (manufactured by Mitsubishi Chemical) is added to 25 l of soaking water that has been employed for soaking shelled soybeans after the manner of Practical Embodiment 1 and stirred for 1 hour to cause the adsorption of the isoflavone compounds. Next, the resin is filtered off and washed with 20% ethanol to remove the phenolic acid, and is eluted with 80% ethanol in order to yield the isoflavone compounds. After drying under reduced pressure, the mass of the isoflavone compounds is 1.1 g. Such isoflavone compounds contain approximately 40% of aglycones.

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Applicant: Kikkoman Corp.

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